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Sources of variability in nicotine and cotinine levels with use of nicotine nasal spray, transdermal nicotine, and cigarette smoking

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Aims Nicotine nasal spray and transdermal nicotine are effective aids to smoking cessation, and are being evaluated for treatment of other medical diseases. Wide variation in levels of nicotine and its metabolite, cotinine, have been observed with such therapies. This study aimed primarily to assess sources of individual variability in nicotine and metabolite plasma levels from these dosing systems and from cigarette smoking.

Methods Twelve cigarette smokers, studied on a clinical research ward, received four treatments of 5 days duration each, including (1) cigarette smoking, 16 cigarettes/day; (2) transdermal nicotine, 15 mg/day; (3) nicotine nasal spray, 24–1 mg doses/day; (4) placebo nicotine nasal spray, 24 doses/day. On a different occasion, the disposition kinetics of nicotine and cotinine were determined via infusion of deuterium-labeled nicotine and cotinine. Plasma levels of nicotine, cotinine, and 3′-hydroxycotinine and daily intake of nicotine during various treatments were examined, as well as pharmacokinetic factors that determined plasma nicotine and cotinine levels.

Results There was considerable individual variation in plasma nicotine and cotinine levels and in the daily dose of nicotine absorbed from various delivery systems, with most variability with nicotine nasal spray (fivefold) and least for transdermal nicotine (two-to threefold). Plasma nicotine levels were determined most strongly by nicotine clearance. Cotinine levels were determined most strongly by dose of nicotine and, to a lesser extent, the clearance of cotinine and fractional conversion of nicotine to cotinine.

Conclusions Plasma levels of nicotine and cotinine produced by nicotine therapies are highly variable, due to both wide variability in individual pharmacokinetics and in dose delivery from the products. To compensate for individual differences in clearance, individualization of nicotine dosing based on therapeutic drug monitoring with comparison to nicotine or cotinine levels during cigarette smoking prior to treatment may be necessary to optimize nicotine therapy. This study also validates a recently proposed method for estimating absolute bioavailability of a drug using drug and metabolite pharmacokinetic data, and presents novel data on plasma levels of the metabolite *trans*-3′-hydroxycotinine in people.

Keywords: nicotine, cotinine, 3-hydroxycotinine, transdermal, nasal spray, cigarette smoking, kinetics, metabolism, bioavailability

Introduction

Nicotine replacement medications are the most widely used pharmacological adjuncts to aid smoking cessation. Numerous studies have found an average twofold enhancement of cessation rates compared with placebo for both nicotine gum and transdermal nicotine [1–3]. However, overall quit rates with nicotine replacement therapy remain modest at 20–30%.

In an effort to improve cessation rates, new nicotine medications have been developed, including nicotine nasal spray. Nicotine nasal spray differs from other commonly marketed nicotine medications in that nicotine is absorbed fairly rapidly from the nose and produces psychological effects that resemble those of cigarette smoking. Nicotine nasal spray may, therefore, work in part as a primary reinforcer, substituting for cigarettes. The efficacy of nicotine nasal spray in smoking cessation has been demonstrated in recent trials, including two trials which suggest that nicotine nasal spray may be more beneficial compared with placebo in more dependent compared with less dependent smokers [4–6]. The success rates with nicotine nasal spray were modest at 18–27%, similar to the success rates for other nicotine replacement therapies.

One possible reason for low success rates is that the level of nicotine delivered by nicotine replacement therapies is too low for some individuals. A dose response has been

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demonstrated for transdermal nicotine systems ranging from 7–21 mg day^{-1} [7], suggesting that certain minimal nicotine levels are needed to optimize efficacy for some individuals. At the same time, studies of transdermal nicotine show wide variability in plasma nicotine levels, even when systems of the same nominal dose are applied [8, 9]. We have previously shown considerable variability in the dose of nicotine actually delivered by transdermal systems of the same nominal dose [9, 10]. Plasma concentration data for nicotine nasal spray, available only from one single dose study, also show wide individual variability [11]. The relative importance of individual variability in nicotine clearance and dose of nicotine delivered by the system is not known.

In this study, we have measured blood levels throughout the day and bioavailability of nicotine in smokers using nicotine nasal spray, with comparison in the same smokers to smoking cigarettes and using transdermal nicotine. The focus of the study was the evaluation of individual variability in nicotine intake from various nicotine delivery vehicles, and the factors predicting nicotine and cotinine levels across nicotine products for individual smokers.

Methods

Subjects

Twelve healthy adult male smokers who had smoked at least 18 cigarettes/day and had serum cotinine levels of 150 ng ml^{-1} or greater were the subjects. Subjects ranged in age from 25–57 (mean age, 38 years), smoked 18–50 cigarettes per day (mean, 27.5), with a history of smoking from 9–35 years (mean, 23 years). The preadmission screening plasma cotinine concentration averaged 372 ng ml^{-1} (range, 179–961 ng ml^{-1}). Subjects were confirmed to be in good health by physical examination, medical history, and routine clinical tests. Written informed consent was obtained from each subject. The study was approved by the Committee on Human Research, at the University of California, San Francisco.

Experimental protocol

Subjects were admitted to the General Clinical Research Center at San Francisco General Hospital for 21 days. They were studied in four treatment blocks, each of 5 days duration. The treatments included (1) cigarette smoking, 16 cigarettes/day spaced at regular intervals over 16 h; (2) transdermal nicotine patches labeled as delivering 15 mg over 16 h; (3) nicotine nasal spray containing 1 mg (two sprays) dosed 24 times a day at 40 min intervals for 16 h; and (4) placebo nasal spray, dosed on the same schedule as active nicotine nasal spray. The sequence of the three nicotine-containing medications was balanced using Latin squares. The fourth treatment was in all cases placebo nicotine nasal spray. This was done so as to avoid the possibility of nicotine intoxication during the study. Nicotine intoxication might be anticipated in subjects who have lost tolerance to the noxious effects of nicotine during placebo treatment and then were reexposed to nicotine in a subsequent treatment. Five days duration for each treatment was decided upon as the time needed to reach a steady state with respect to the pharmacokinetics of nicotine and its metabolites with use of the different dosing systems.

Subjects smoked cigarettes of their own choice and were instructed to smoke their cigarettes as they usually smoke them. The transdermal nicotine system for 16 h dosing and the nicotine nasal spray and its placebo were obtained from Pharmacia & Upjohn Consumer Pharmaceuticals (Helsingborg, Sweden). The doses of transdermal patch and spray were chosen to be comparable with the estimated intake of nicotine from smoking 16 cigarettes (based on a typical intake of 1 mg per cigarette) [12]. Subjects received detailed instructions on the proper use of nicotine nasal spray. Prior to the study, each subject was shown a videotape that explained and demonstrated the use of the spray. After being shown how to prime the spray mechanism, subjects were instructed to spray one dose into each nostril. They were instructed not to sniff or blow the nose for several minutes. The first few doses were taken under direct supervision, with reminders on how to properly use the spray. Initially, the nasal spray was uniformly noxious, with subjects describing burning of the nose and throat and watery eyes. Tolerance developed rapidly to these effects so by the second or third day doses were tolerated without difficulty. On day 5 of each treatment block, blood samples (5 ml each) were taken at 4 h intervals from an indwelling venous catheter for measurement of plasma concentrations of nicotine, cotinine, and *trans*-3′-hydroxycotinine and blood carboxyhaemoglobin levels. Samples were drawn midway between cigarette or nicotine nasal spray doses. The timing of blood samples was such that peak levels after smoking or nasal spray would have been missed, so assessment of nicotine intake was estimated based both on nicotine and cotinine levels, as described below.

On a separate occasion, either before or after the main study, subjects participated in a study in which their systemic pharmacokinetics of nicotine and cotinine were determined. This study, as described previously, included a 30 min infusion of deuterium-labeled nicotine and cotinine with subsequent blood sampling for 96 h [13].

Analytical chemistry

Plasma concentrations of nicotine and cotinine in the main study were determined by gas chromatography with nitrogen-phosphorus detection, as described elsewhere [14–16]. Concentrations of *trans*-3′-hydroxycotinine were determined by gas chromatography-mass spectrometry [15]. The interday coefficient of variation for trans-3′-hydroxycotinine was 1–13% over a concentration range of 5–500 ng ml^{-1}. Concentrations of deuterium-labeled nicotine and cotinine were measured by gas chromatography-mass spectrometry with the use of nicotine-3′,3′-d_2-N′-methyl-d_2-nicotine (nicotine-d_4) and cotinine-2,4,5,6,4′-4′-d_6-N′-methyl-d_3 (cotinine-d_9) as internal standards, as described previously [13, 16]. Intraday and interday coefficient of variation for nicotine-d_2 were 2–5% over a concentration of 1–100 ng ml^{-1}; the coefficient of variation for cotinine-d_2 and cotinine-d_4 ranged from 3–12% over a concentration range of 10–1000 ng ml^{-1}. Concentrations of nicotine-d_2 and cotinine-d_2 were corrected for the presence of naturally occurring stable isotopes in nicotine from tobacco. The

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syntheses of nicotine-d_2 and cotinine-d_4 for the infusion study have been described previously [13, 17].

Data analysis

Plasma nicotine and cotinine concentration data from the intravenous infusion study were analyzed using model independent methods [18]. The clearance of nicotine, clearance of cotinine, as well as fractional conversion of nicotine to cotinine were determined as described previously [13].

The 24 h dose of nicotine (D) systematically absorbed from nicotine nasal spray, cigarette smoking or transdermal nicotine was determined by two different methods. The first used the area under the plasma concentration time curve, AUC, for natural nicotine (AUC_{nic}) and the average clearance of labeled nicotine (CL_{nic-d_2}) according to the equation

$$D = AUC_{nic} \times CL_{nic-d_2}$$

AUC_{nic} over 24 h was measured by the trapezoidal method. The estimation of dose assumed that the AUC_{nic} represents a steady state value. Since nicotine has a half-life of 2 to 3 h and subjects had been using particular forms of nicotine delivery systems for 5 days, steady state is assumed.

The 24 h intake of nicotine was also estimated using the 24 h average plasma cotinine concentration at steady state ($\bar{C}_{cot}$) along with the clearance of cotinine-d_4 (CL_{cot-d_4}) and the fractional conversion of nicotine to cotinine (f_c) by the equation

$$D = \frac{\bar{C}_{cot} \times CL_{cot-d_4}}{f_c}.$$

The derivation of this equation is described in a recent publication [13]. By using the daily intake of nicotine and number of cigarettes smoked per day or the number of doses of nicotine nasal spray, an average nicotine intake per dose delivery system was also estimated. The values for 24 h dose of nicotine estimated based on nicotine vs. cotinine levels in the three conditions were compared by repeated measures analysis of variance and by the Bland & Altman method [19].

Factors that determine average plasma levels of nicotine and cotinine during the day with use of various nicotine delivery systems were examined. Simple and multiple linear regression analyses were used to determine the relationships between systemic dose of nicotine, clearance of nicotine and cotinine, and fractional conversion of nicotine and average plasma concentrations of nicotine and cotinine. Average plasma nicotine and cotinine concentrations between 12 noon and 12 midnight (mean of four concentrations) were analyzed because these concentrations approximate plateau levels across the time interval, and these are times that are most likely to be used for therapeutic drug monitoring, if that were to be performed. The data from the transdermal nicotine session only along with data from a large clinical trial of transdermal nicotine have been used in a similar analysis in another publication [20].

Results

Plasma concentrations of nicotine, cotinine, and *trans*-3′-hydroxycotinine throughout the day in the various treatment conditions are shown in Figure 1. Average concentrations of nicotine and metabolites were similar for nicotine nasal spray and transdermal nicotine conditions, while levels were on average twice as high during cigarette smoking. Individual subject values for plasma concentrations of nicotine and cotinine averaged from 12 noon to 12 midnight (mean of

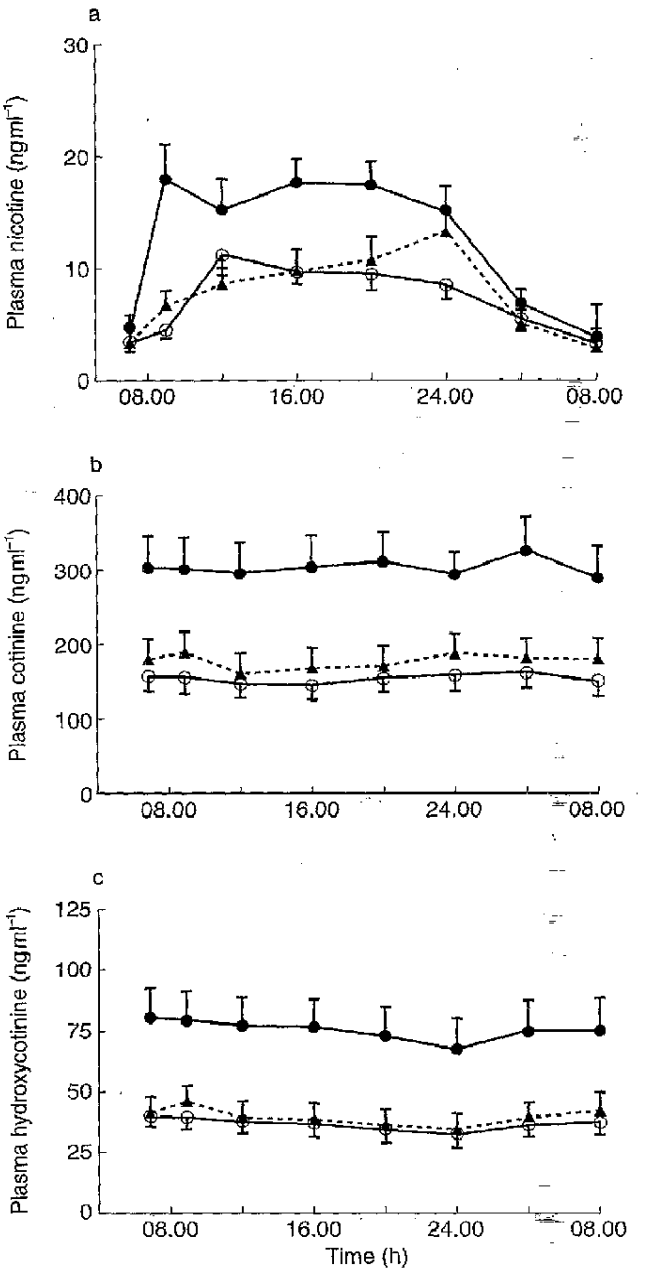

Figure 1 Plasma nicotine (a), cotinine (b), and *trans*-3′-hydroxycotinine (c) concentrations over 24 h on the fifth day of nicotine dosing via cigarette smoking (16 cigarettes/day, ●), transdermal nicotine (15 mg/16 h patch, ○), and nicotine nasal spray (24 doses/day, ▲). Vertical bars indicate s.e.mean, $n = 12$.

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four values) are shown in Figure 2; average values are recorded in Table 1. The individual variability in plasma nicotine and cotinine levels was similar for cigarette smoking and transdermal nicotine (coefficient of variation, ~40%) but was higher for nicotine nasal spray (coefficients of variation, 62% and 56% for plasma nicotine and cotinine, respectively).

Clearance values and fractional conversion of nicotine to cotinine (derived from infusions of labeled nicotine and cotinine) are shown in Table 2. The daily systemic doses of nicotine during the various treatment conditions are shown in Table 3. The mean values of estimates of daily nicotine dose determined using the AUC_{nic} method and the 24 h average cotinine method were quite close. Repeated measures analysis of variance revealed no significant differences for the dose estimation method and no significant condition X method interactions. Analysis by the Bland & Altman technique revealed no relationship between the differences in the value of the estimates of nicotine dose by the two methods and the mean value of the estimates *per se*, indicating that these methods can be used interchangeably [19]. Because there is likely to be more variability in the AUC nicotine method due to temporal fluctuations of plasma nicotine level after individual cigarettes and doses of nicotine nasal spray, the average cotinine method was used as the basis for daily nicotine dose for subsequent analyses. The daily dose of nicotine was similar for nicotine nasal spray, averaging 13.2 mg day^{-1}, and transdermal nicotine, averaging 11.2 mg day^{-1}. The daily intake from cigarette smoking averaged 22.9 mg (Table 3).

Individual variability in 24 h intake of nicotine per unit delivery system is shown in Figure 3. The average intake of nicotine was 0.55 mg per dose (two sprays) of nicotine nasal spray, 1.43 mg per cigarette, and 11.2 mg per transdermal nicotine system. There was considerable variability in nicotine intake per dose for all systems, with a range of 0.21–1.07 mg (CV 49%, 95% CI 0.38–0.72) for nicotine nasal spray, 0.65–2.1 mg (CV 35%, 95% CI 1.11–1.76) for cigarette smoking, and 8.8–16.9 mg (CV 22%, 95% CI 9.1–17.3) for transdermal nicotine. The average plasma cotinine concentration per cigarette smoked during the study was 18.9 ng ml^{-1} per cigarette (s.d., 8.6).

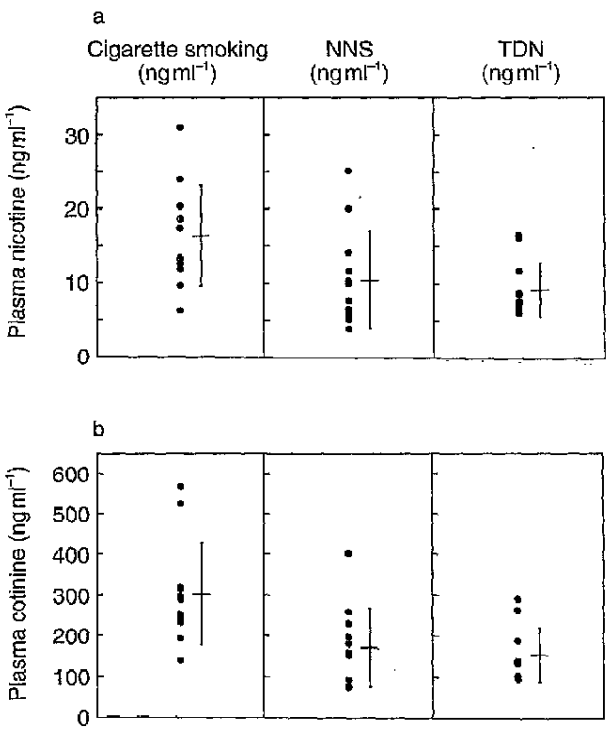

Figure 2 Individual values for average plasma nicotine (a) and cotinine (b) concentrations during various nicotine treatment conditions. Individual values represent the mean of levels measured at 12.00, 16.00, 20.00, and 24.00 h on day 5 of each treatment condition. Horizontal bars indicate mean (±s.d.).

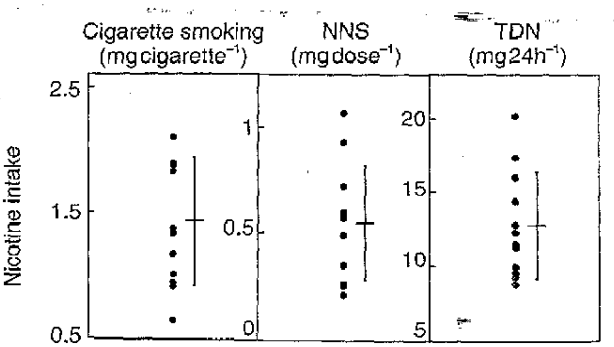

Figure 3 Individual values for intake of nicotine per unit delivery system. Values are expressed as nicotine per cigarette, nicotine per nasal spray dose (consisting of two sprays, one in each nostril), and nicotine per patch, based on 24 h intake on day 5 of nicotine treatment. Horizontal bars indicate mean (±s.d.).

Table 1 Daytime average‡ plasma nicotine, cotinine and 3′-hydroxycotinine concentrations in different nicotine treatment conditions.

	Cigarette smoking			*Nicotine nasal spray*			*Transdermal nicotine*		
	Nicotine	*Cotinine*	*3HC*	*Nicotine*	*Cotinine*	*3HC*	*Nicotine*	*Cotinine*	*3HC*
Mean* (ng ml^{-1})	16.3	301	73	10.5	170	37	9.2	152	35
s.d.	6.7	125	42	6.5	95	23	3.6	66	18
Range	6.3–30.9	139–567	19–131	3.9–25.2	73–401	7–83	6.1–16.5	95–291	12–66
95% C.I.	12.1–20.6	221–381	47–100	6.4–14.6	109–231	23–52	6.9–11.5	111–196	24–47
CV† (%)	41.1	41.5	57.5	61.9	55.8	62.1	37.9	42.8	51.4

*Mean of average daytime values for 12 subjects.
†CV = Coefficient of variation.
‡Average value for each subject based on plasma levels at 12.00, 16.00, 20.00, and 24.00 hs.

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Table 2 Pharmacokinetics of nicotine and cotinine, measured by infusions of deuterium-labeled nicotine and cotinine.

Subject	Body weight (kg)	Nicotine clearance (ml min^{-1})	Cotinine clearance (ml min^{-1})	Fractional conversion nicotine to cotinine (f_c)	K* ($\times 10^{-3}$)
1	67.6	949	23	0.61	55.3
2	68.5	1465	53	0.76	101.6
3	90.6	1206	53	0.86	89.1
4	86.4	1363	44	0.84	75.8
5	63.3	565	21	0.61	51.5
6	58.4	1086	47	0.82	83.4
7	88.7	1236	54	0.82	96.9
8	80.5	1533	51	0.88	83.5
9	75.6	1661	74	1.01	105.9
10	117.9	1285	49	0.81	88.8
11	76.1	785	27	0.71	55.2
12	76.2	1643	38	0.88	63.3
Mean	79.2	1231	45	0.80	79.2
s.d.	15.8	339	15	0.11	18.9

*Conversion factor based on equation: D_{nic}(mg 24 h^{-1}) = plasma$_{cot}$(ng ml^{-1}) × K;

$$\left(K = \frac{CL_{cot}}{f_c}(\text{ml/min}) \times 1.44 \times 10^{-3}\right)$$

(see reference 13 for derivation).

Table 3 Nicotine intake during cigarette smoking, nicotine nasal spray and transdermal nicotine treatment.

	Cigarette smoking		Nicotine nasal spray		Transdermal nicotine	
Subject	AUC[a] method (mg)	COT[b] method (mg)	AUC[a] method (mg)	COT[b] method (mg)	AUC[a] method (mg)	COT[b] method (mg)
1	21.2	14.5	8.1	5.2	17.5	14.5
2	22.3	29.0	23.1	25.7	20.3	13.6
3	17.9	21.4	13.9	13.8	9.6	9.1
4	11.1	10.5	8.7	12.0	14.4	10.6
5	20.8	16.2	17.6	11.9	12.3	10.4
6	14.9	15.1	7.6	6.4	8.9	8.8
7	29.6	30.3	14.0	17.5	9.3	9.5
8	16.8	18.7	7.2	6.0	12.8	11.0
9	24.1	30.0	10.0	8.4	11.3	9.4
10	27.3	22.0	10.3	14.2	10.0	9.1
11	24.0	33.7	18.7	12.4	11.5	16.9
12	38.7	33.7	21.2	14.6	16.0	11.7
Mean	22.4	22.9	13.4	13.2	12.8	11.2
s.d.	7.3	8.1	5.6	6.4	3.6	2.5
CV (%)	32.6	35.4	41.8	48.5	28.1	22.3

[a]AUC method: Dose based on 24 h area under plasma nicotine concentration curve and nicotine clearance.
[b]COT method: Dose based on 24 h average plasma cotinine concentration and K value (see Table 2).

as compared with 15.0 ng ml^{-1} per cigarette (s.d., 11.9) based on preadmission cotinine level and cigarette consumption.

An attempt was made to predict average nicotine or cotinine concentrations in various treatment conditions using individual values for a systemic dose of nicotine and individual clearance values for nicotine and cotinine and fractional conversion of cotinine. A multiple regression was performed according to the general equations PNC = f (D_{nic}, CL_{nic}) and PCC = f(D_{nic}, CL_{nic}, CL_{cot}, f_c), where PNC = average plasma nicotine concentration, PCC = average plasma cotinine concentration, f_c = fractional

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conversion of nicotine to cotinine. Regression equations for the various products were as follows:

Cigarette smoking:

$$PNC = 28.6 - 17.7CL_{nic} + 0.41D_{nic};\ r^2 = 0.73$$

$$PCC = -88 - 57CL_{nic} - 7604CL_{cot} + 593f_c + 14.3D_{nic};\ r^2 = 0.99$$

Nicotine nasal spray:

$$PNC = 19.4 - 12.7CL_{nic} + 0.50D_{nic};\ r^2 = 0.65$$

$$PCC = -160 - 81CL_{nic} - 4958CL_{cot} + 588f_c + 13.8D_{nic};\ r^2 = 0.95$$

Nicotine patch:

$$PNC = 12.8 - 7.05CL_{nic} + 0.46D_{nic};\ r^2 = 0.59$$

$$PCC = -52 - 33\ CL_{nic} - 2987CL_{cot} + 286f_c + 17.1D_{nic};\ r^2 = 0.95$$

In predicting plasma nicotine concentrations, multiple regression indicated that clearance of nicotine accounted for more variance in all treatment conditions (Table 4). For plasma cotinine concentrations, the order of entry into the regression was similar for all treatments: Dose of nicotine, clearance of cotinine, fractional clearance of nicotine to cotinine, and clearance of nicotine (Table 4).

Discussion

This study provides novel information in several respects. (1) A recently proposed method of estimating daily nicotine dose using average cotinine concentrations and nicotine pharmacokinetic data is validated; (2) this is the first study determining systemic absorption of nicotine from nicotine nasal spray during regular daily use; (3) a detailed analysis of individual variability in nicotine intake from various nicotine delivery systems under carefully controlled dosing conditions is presented; (4) the relative importance of dose *vs* individual pharmacokinetic characteristics in determining blood levels of nicotine and cotinine during regular use of several nicotine delivery systems is examined, and (5) novel data on plasma *trans*-3′-hydroxycotinine concentrations during different nicotine treatment conditions are presented.

Table 4 Multiple regression — Predictors of average plasma nicotine and plasma cotinine concentrations†.

	Cigarette smoking Δr^2	*Nicotine nasal spray* Δr^2	*Transdermal nicotine* Δr^2
Plasma nicotine concentration			
CL_{nic}	0.55*	0.46*	0.57*
D_{nic}	0.23*	0.25*	0.09
Plasma cotinine concentration			
D_{nic}	0.64*	0.73*	0.77*
CL_{cot}	0.30*	0.15*	0.16*
f_c	0.05*	0.06*	0.02
CL_{nic}	0.007*	0.02	0.007

*$P<0.05$ for entry into regression.

†Mean of four measurements at 12.00, 16.00, 20.00, and 24.00 h.

The intent of our study design was to simulate typical daily exposure to cigarette smoke, nicotine nasal spray, and transdermal nicotine, while controlling dosing schedules in order to compare cardiovascular effects, which will be reported elsewhere. The selection of 16 cigarettes/day and 24 nicotine spray doses/day was to provide similar nicotine doses from cigarette smoking compared with the standard transdermal delivery of 15 mg/day. Sixteen cigarettes/day is less than that usually smoked by our subjects. Of note is that the average intake of nicotine per cigarette was 1.4 mg, which is higher than the typical 1 mg per cigarette, which was the basis for our original projection [12]. The use of 24 nicotine spray doses (each dose representing two sprays, one in each nostril) is similar or a little greater than the number of doses taken by smokers during smoking cessation trials [4–6].

As anticipated, the daily dose of nicotine from nicotine nasal spray and transdermal nicotine were similar, although the nicotine patch dose was a little lower than the 15 mg determined from a previous study [10]. Treatment for 5 days in each condition is relevant because 5 days allows adequate time to achieve steady daily levels of nicotine and its metabolites (as confirmed by comparing the 08.00 h values at the beginning and the end of the blood sampling day, seen in Figure 1) and to allow time to become practiced in the use of nicotine nasal spray. Initially, nicotine nasal spray was very irritating to the nose and throat and caused watery eyes, coughing and/or sneezing. However, tolerance developed quickly to these noxious effects, so that by day 2 or 3 all doses could be taken without difficulty. By day 5, nicotine nasal spray was easily and consistently dosed by all subjects.

Because we had characterized individual pharmacokinetic parameters as well as measuring plasma nicotine and plasma cotinine concentrations over 24 h during various treatment conditions, we were able to validate a recently proposed method for estimating daily intake of nicotine [13]. The usual method of estimating a systemic dose of a drug is to measure the area under the plasma concentration time curve and the clearance (termed as the AUC method). Our new method involved measuring an average plasma cotinine concentration and using an individual's data on fractional conversion of nicotine to cotinine and clearance of cotinine (the latter converted into a 'K' factor). In the present study, nicotine intake by these two methods was highly correlated and produced very similar average estimates.

One assumption behind both dose estimation methods is that the clearance of nicotine and cotinine and fractional conversion of nicotine to cotinine are similar on the intravenous study day to that operative during the various treatment conditions. We measured nicotine and cotinine pharmacokinetics using an intravenous infusion protocol at a different time from the study treatments. Our unpublished data indicate that pharmacokinetic values for an individual remain fairly constant on repeated measurement over time. Also, as observed in this study, clearance values were strong predictors of plasma nicotine and cotinine concentrations in various treatments. This would suggest that clearance values

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measured at a distant time were, in fact, operative during the study treatments. Thus, we consider the assumption that clearance values derived from intravenous studies are similar to those during treatment conditions to be a reasonable one.

This is the first study to examine the systemic dose of nicotine from nicotine nasal spray during regular daily dosing conditions. Our estimate of 0.55 mg per dose is very similar to that obtained from single doses of nicotine nasal spray studied in eight subjects [11]. The estimate of the systemic dose of nicotine from transdermal nicotine was lower than that reported in a previous study of the same patch in our laboratory [10]. As noted previously, the nicotine intake from cigarette smoking in this study was greater than the typical 1 mg/cigarette dose reported in other studies of *ad libitum* smoking [12]. Our subjects had smoked an average of 27 cigarettes/day prior to entering the study. Thus, our subjects' consumption of cigarettes was restricted compared with their usual consumption. Smokers smoke cigarettes more intensively when their access to cigarettes is restricted, reflecting the tendency to regulate daily intake of nicotine [21]. More intensive smoking was demonstrated in the present study by the higher cotinine per cigarette values during the experimental smoking treatment compared with preadmission screening values. Thus, nicotine regulation behaviour most likely explains why the intake of nicotine per cigarette in our study was higher than anticipated.

There was marked individual variability in the plasma levels of nicotine and cotinine as well as variability in the systemic dose of nicotine in the various treatment conditions. Individual variability in plasma nicotine concentration is important because nicotine is the therapeutic principle of nicotine replacement therapy and nicotine levels are likely to determine, at least in part, efficacy and toxicity. Cotinine levels are of interest because cotinine has been widely used as a biomarker of nicotine intake from tobacco and has been suggested as a way to guide nicotine replacement therapy [22]. A four- to fivefold variability in nicotine and cotinine plasma concentrations during cigarette smoking, despite controlling the number at 16 per day, is not surprising because it is known that people smoke cigarettes differently from one another, taking different numbers of puffs, taking different depths and duration of puffs, etc. This study shows that the extent of variability in nicotine and cotinine levels is as great or greater with nicotine nasal spray than with cigarette smoking, and is substantial (threefold) with transdermal nicotine dosing, despite all subjects receiving the same dose patch.

There was considerable variability in systemic doses of nicotine delivered by all delivery systems. The greatest variability was seen with nicotine nasal spray (fivefold range, coefficient of variation of 48%), intermediate with cigarette smoking (threefold range, CV 35%), and lowest with transdermal nicotine (twofold range, CV 22%).

The high degree of individual variability in dose absorbed from nicotine nasal spray may be explained by several factors, including uncertainty in the volume sprayed (due to not pressing the injector completely and/or not priming the injector completely), loss of some spray by rapid passage into the nasopharynx, or loss due to sneezing, and/or to individual differences in nasal absorption. We have no data on variability from dose to dose within an individual. But it is clear that the average dose of nicotine can vary substantially from person to person.

The variability in systemic dose of transdermal nicotine was also substantial, although less than that from other delivery systems. Variability in systemic absorption of nicotine from the nicotine patches has been observed in prior studies [9, 10]. The reason for such variability is presumably differences in the extent of release of nicotine from the patch and/or rate of transdermal absorption. Thus, even when a patch of a known strength is administered, the systemic dose cannot be assumed with accuracy better than a twofold range.

Multiple regression analysis allowed us to compare the relative contributions of dose and individual pharmacokinetics as determinants of plasma nicotine and cotinine concentrations in various treatments. Plasma nicotine concentrations during cigarette smoking and nicotine treatments were best predicted by nicotine clearance (46–57% of variance) and to a lower extent by the dose of nicotine (additional 9–23% variance). In contrast, for plasma cotinine, the dose of nicotine was the most important predictor (64–77% of variance), clearance of cotinine next most important (15–30% of variance), while fractional conversion of nicotine to cotinine and clearance of nicotine contributed relatively little. Thus, our analysis indicates the major source of individual variation in plasma nicotine concentrations is individual differences in the clearance of nicotine. The dose of nicotine is an important determinant of nicotine levels, but is less important than individual pharmacokinetic characteristics. Dose of nicotine is the major source of individual variation in plasma cotinine levels, with cotinine clearance explaining a significant but lesser degree of the variance.

We present herein the first data on plasma *trans*-3′-hydroxycotinine concentrations during regular cigarette smoking, as well as during the use of nicotine medications. *Trans*-3′-hydroxycotinine is the major metabolite of cotinine, and in the urine is the single most abundant metabolite of nicotine [23–26]. *Trans*-3′-hydroxycotinine is believed to be conjugated by *O*-glucuronidation, while nicotine and cotinine are conjugated by *N*-glucuronidation [26, 27]. Plasma levels reported are those of unconjugated drug only. Plasma *trans*-3′-hydroxycotinine levels were, in general, 25–28% those of cotinine levels, although there was considerable variability in the ratio (range 0.08–0.56). Across individuals, 3′-hydroxycotinine levels were not significantly correlated with cotinine levels, consistent with individual variability in the extent of metabolism of cotinine to *trans*-3′-hydroxycotinine [26]. *Trans*-3′-hydroxycotinine has been suggested as a possible biomarker of nicotine exposure, but its utility is likely to be primarily as a urine measurement, because in the urine it represents the greatest percentage of nicotine dose.

The clinical implications of our study are several. First, plasma nicotine concentrations are substantially lower with nicotine nasal spray and transdermal nicotine compared with cigarette smoking, even when cigarettes are restricted to 16 per day. If replacement of a daily dose of nicotine with an amount comparable to that derived from cigarette smoking is desirable, much larger doses of transdermal nicotine and

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nicotine nasal spray will be needed. This may be the case with the transdermal nicotine, as some recent studies indicate better results with more dependent smokers when higher doses of transdermal nicotine are administered [28, 29]. On the other hand, nicotine nasal spray appears to work better compared with placebo in more dependent rather than less dependent smokers [4, 5]. This effect in highly dependent smokers is seen despite relatively low plasma nicotine concentration compared to smoking. This observation is consistent with the idea that rapid absorption of nicotine, producing higher transient nicotine levels in arterial blood and the brain, is much more effective in reducing urges to smoke and perhaps providing other positive reinforcement compared with slower release preparations [30]. (Note that rapid absorption of nicotine from nasal spray was not shown in the present study because of the blood sampling schedule, but has been shown by other researchers who used more intensive sampling [11].)

The wide individual variability in plasma levels of nicotine and cotinine and in dose delivery from nicotine products may have important implications in trying to optimize nicotine replacement therapy. These data make it clear that there is no way to accurately predict what level of nicotine and cotinine will be produced in an individual by a particular dose of nicotine replacement therapy. This is because of wide variability in both individual pharmacokinetics and dose delivery by the products. Nicotine has a relatively narrow therapeutic-toxic window. Excessive doses produce nausea and vomiting and other physiological disturbances. Therefore, individualization of nicotine dosing based on therapeutic drug monitoring may be necessary to optimize nicotine replacement therapies. The observation that clearance is more important than dose in determining the plasma level of nicotine suggests that the best approach to dose optimization should be matching blood levels to levels measured during *ad libitum* cigarette smoking prior to treatment. This can be done by selecting a particular percentage of 'replacement' of nicotine compared to cigarette smoking, and using that percentage to adjust levels of nicotine or cotinine during nicotine replacement therapy. Such a replacement strategy eliminates the problem of individual variability in clearance because the same clearance operates to determine nicotine and cotinine levels during nicotine therapy as during cigarette smoking. The approach of prospective matching of cotinine levels has been attempted in one study, which shows considerable promise in enhancing cessation rates above those commonly reported with recommended nicotine replacement therapy dosing schedules [29].

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